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Procedia Engineering 56 (2013) 772 – 776

Procedia Engineering

www.elsevier.com/locate/procedia

5th BSME International Conference on Thermal Engineering

Tailoring the molecular distribution of bioreactive chemical groups to modulate adhesion to soft tissues

Jahid Ferdous[a], Eva Juarez-Perez[a], Tarek Shazly[a,b,*]

[a]*Biomedical Engineering Program, College of Engineering and Computing, University of South Carolina, Columbia, SC – 29208, USA*
[b]*Department of Mechanical Engineering, College of Engineering and Computing, University of South Carolina, Columbia, SC – 29208, USA*

Abstract

The application of adhesive materials can significantly augment available wound repair techniques and improve healing following surgical interventions. However, soft tissue surface chemistry and mechanical loading conditions significantly vary among potential applications and confound the development of a universal adhesive material with acceptable clinical performance. Current materials are however not designed on a tissue-specific basis and as a result, force a choice between high adhesion strength and biocompatibility. We hypothesize that rational tuning of bioreactive chemical groups distribution along adhesive polymer chains can facilitate optimal tissue-material interactions without inducing a concomitant reduction in material biocompatibility. A series of two-component, aldehyde-mediated adhesive materials are synthesized using dextran aldehyde and chitosan polymers to achieve both cohesive and adhesive cross-linking through the formation of imine bonds. Within the material series, the distribution of bioreactive aldehyde groups along the constituent polymer chains are varied while keeping the total aldehyde content at a constant level. The gelation time, elastic modulus, and adhesion strength to renal artery tissue surfaces are measured for each material formulation. Results show that the reactive chemical groups distribution strongly modulates adhesive interactions. Optimizing material bioreactive groups distribution in a tissue-specific manner could provide a means to circumvent the persistent tradeoff between adhesion and biocompatibility.

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Keywords: Adhesive Materials; adhesion; biocompatibility; molecular distribution; soft tissue.

1. Introduction

Soft tissue adhesive materials assist to attain homeostasis, prevent anastomatic leakage, provide mechanical reinforcement to compromised tissue, deliver therapeutic compounds , and thus facilitate tissue healing at surgical sites [1]. Although clinical outcomes of commercially available adhesive materials, such as cyanocrylate and fibrin, are usually satisfactory, they are still limited by biocompatibility and the degree of adhesion, respectively. Highly reactive cyanocrylate strongly adheres with the soft tissue but generates toxic degradation by-products, whereas highly biocompatible fibrin only loosely adheres to tissue surfaces [2-3]. Surface chemistry variation among soft tissues, such as heart, lung, kidney, intestine, and duodenum etc., and diverse mechanical loading conditions, also preclude favorable clinical outcomes in many potential indications for adhesive materials.

Among different strategies to develop effective adhesive materials in recent past, hydrogel-based approaches have attracted more attention due to the potential to tune material properties to optimize material properties, including adhesion strength, degradation kinetics, bulk modulus, and cytocompatibility [4]. Previous studies have shown that aldehyde-

* Corresponding author. Tel.: +1-803-777-4678 ; fax: +1-803-777-0106.
E-mail address: SHAZLY@cec.sc.edu

Selection and peer review under responsibility of the Bangladesh Society of Mechanical Engineers
doi:10.1016/j.proeng.2013.03.193

mediated materials show potential to control adhesive interactions via titration of free aldehyde groups content [5]. Moreover, tissue-specific adhesion has been demonstrated via adjustment of material aldehyde content to match the amine groups available on a targeted tissue surface [6]. Herein we postulate that tissue-material adhesion can be further controlled through tuning of the aldehyde groups distribution along constituent polymer chains – because this can be done at a fixed level of overall aldehyde groups content, the proposed strategy is anticipated to maintain material biocompatibility.

In this study, a family of adhesive materials having four different aldehyde groups distributions is synthesized and characterized. The total number of aldehyde groups was kept constant among formulations to ensure that property variations were not a consequence of net material reactivity, but rather due to the presentation of reactive groups along polymer chains. Results indicate the gel formation time does not change significantly until the spacing between two aldehyde groups reaches the threshold value. Moreover, a stiffer material is formed when distance among aldehyde groups is small. Tissue-material adhesive interactions with porcine renal arteries reveal that adhesive strength is also modulated by the aldehyde groups distribution, although in a complex manner that warrants further investigation. Our results provide preliminary indication that rationale tuning of the bioreactive chemical groups distribution can provide a basis for optimization of tissue-specific adhesive materials.

2. Materials and methods

2.1. Material synthesis

A series of two-component, aldehyde-mediated adhesive materials were synthesized using dextran aldehyde and chitosan polymers. A 10 wt.% aqueous solution of dextran with an average molecular weight of 40 kDa was mixed with a 5-15 wt.% solution of sodium periodate at room temperature to yield dextran aldehyde with different oxidation levels, herein defined as the percent of oxidized glucose rings measured via acid-base titration (Fig 1a). The solution was then dialyzed, lyophilized, and reconstituted to yield an aqueous polymer solution of dextran aldehyde. A 2 wt% aqueous solution of chitosan with an average molecular weight of 340 kDa was also prepared, providing a cohesive material component whose amine groups can chemically cross-link with dextran aldehyde through imine bond formation. Material components (dextran aldehyde and chitosan) were loaded into and delivered with a dual-chamber syringe equipped with a 12-step mixing tip, enabling repeatable component mixing and material network formation (Figure 2a). The average distance between rings containing aldehyde groups in terms of the monomer repeating unit is defined as the distribution index, and systematically varies among material formulations. A constant number of aldehyde groups and ratio between aldehyde to amine groups were maintained through adjusting the dextran aldehyde solid content prior to material formation (Table 1).

2.2. Gelation time

Constant volumes of dextran aldehyde and chitosan solutions were injected using dual-chamber syringe into a 24 well culture plate where a magnetic stir rod was rotating at a predefined fixed speed. The time to form a solid globule around the magnetic stir rod by visual inspection was considered as the gelation time. Results are presented as the average and standard deviations of three independent readings for each material compositions.

Table 1. Composition descriptions of the investigated dextran aldehyde:chitosan material series

Composition	Dextran Aldehyde					Chitosan			Dextran aldehyde:Chitosan
	Molecular Weight (kDa)	Percent Oxidation (%)	Solid Content (%)	Distribution Index	Aldehyde Content (# per ml)	Molecular Weight (kDa)	Solid Content (%)	Amine Content (# per ml)	Reactive Groups Ratio (CHO:NH_2)
A	40	19.44	13.25	5.14	2.30×10^{20}	340	2	7.63×10^{19}	3
B	40	24.30	10.80	4.11	2.30×10^{20}	340	2	7.63×10^{19}	3
C	40	42.12	6.30	2.37	2.30×10^{20}	340	2	7.63×10^{19}	3
D	40	71.28	3.59	1.40	2.30×10^{20}	340	2	7.63×10^{19}	3

2.3. Compressive elastic modulus (E_c)

Cylindrical samples of the materials were prepared using silicon mold with a diameter and height of 9.5 mm and 6.25 mm, respectively, and subsequently used to measure the compressive elastic modulus (E_c). Uniaxial tensile testing (Bose® Biodynamic Test Instrument, Minnetonka, MN) was used to apply ramped uniaxial compressive displacement (maximum 30% of initial samples' height with a rate of 0.005 mm/sec). Sample forces and displacements data were continuously recorded at a data acquisition rate of 1.67 points/sec using a software package (Wintest® Software, Minnetonka, MN). Force and displacement data were transformed into true stress and strain for each material composition to calculate E_c. Results are presented as the average and standard deviations of three independent readings for each material compositions.

2.4. Adhesion strength

Fresh intact porcine kidneys were collected from local slaughter house and stored in the normal saline at low temperature before dissection. Renal arteries were carefully separated from the kidneys and attached with the connectors of uniaxial tensile testing (Bose® Biodynamic Test Instrument, Minnetonka, MN) using parafilm in such a way that the adventitia layer were placed outside and formed a flat surface (Fig 3b). A controlled volume of adhesive material was injected into one tissue surface followed by immediate attachment with the other surface under specified initial compressive load. A constant curing time was maintained for all the samples prior to mechanical testing. Ramped uniaxial tensile displacement (maximum 5 mm with rate of 0.005 mm/sec) was applied and corresponding force and displacements data were recorded using a software package (Wintest® Software, Minnetonka, MN). The maximum load prior to tissue-material interfacial failure was recorded and reported as the maximum adhesion strength. Results are presented as the average and standard deviations of four independent readings for each material compositions.

3. Results and discussion

Dextran aldehyde oxidation level is directly dependent on the amount of oxidizing agent (sodium periodate) (Fig 1b). Prolonged mixing between dextran and sodium periodate ensures the conversion of dextran into dextran aldehyde with no intermediate products. More than 50% glucose chains of 40 kDa dextran open and proportionally the number of reactive aldehyde groups increase when 15 wt% sodium periodate was used in comparison to 5 wt%. The molecular weight of the oxidized polymer is a function of the level of oxidation. Moreover, for a fixed amount of sodium periodate, the initial dextran molecular weight controls the level of oxidation (data not shown).

Alternation in aldehyde groups distribution results in similar gel formation time among materials except for in case A, which has maximum solid content with minimum oxidation level (Fig 2b). The gelation time can be easily lowered by increasing the solid content to achieve a gelation time more suitable for surgical applications. In general, higher solid content and percent oxidation promote network formation through increasing the possibility of cohesive chemical interactions with chitosan amine groups [7]. However, case A having the maximum aldehyde groups distribution index results in almost 3-fold higher gelation time compared to the other formulations. This suggests that a critical aldehyde groups distribution index may exist (between 5.14 and 4.11) above which material cross-linking is depressed due to steric inhibition, as further supported by the observed reduction in material stiffness at higher distribution indices (Fig 2c). Insignificant elastic modulus change is observed when the aldehyde groups distribution index is below 2.37.

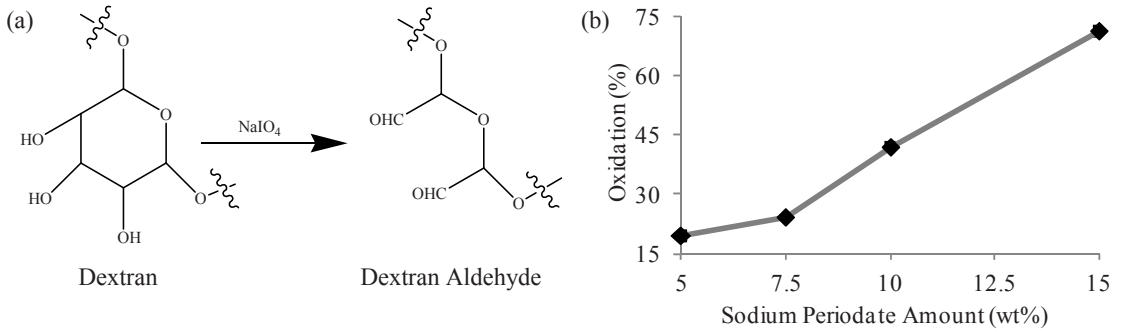

Fig. 1. (a) Sodium periodate oxidizes the dextran glucose ring and results two aldehyde groups; (b) Higher amount of sodium periodate opens more glucose rings and increases the oxidation level of dextran aldehyde.

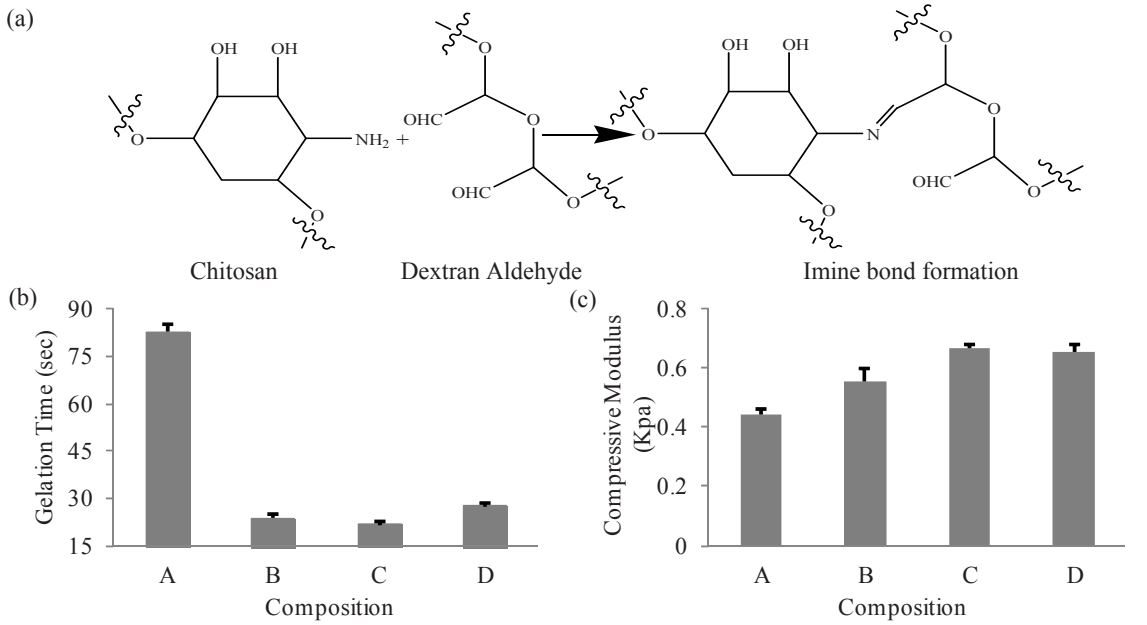

Fig. 2. (a) Imine bond formation between chitosan and dextran aldehyde; (b) Aldehyde groups distribution index affects the gelation time only when it is above the critical value; (c) Material stiffness increases when average distance between two open glucose rings decreases.

The adhesive strength of the material to the tissue surface depends on imine bond between the aldehyde groups of the dextran aldehyde and amine groups of tissue-present proteins (Fig 3a), similar in principle to the formation of internal material cross-linking (Fig 2a). When tissue-material constructs are placed in tension, all formulations underwent cohesive failure indicating that tissue-material cross-linking exceeds internal network formation. Adhesive variation with distribution index indicates that intermediate spacing diminishes adhesive strength compare to distributions at the ends of the examined range. This non-monotonic response suggests the existence of multiple reactive groups distributions that promote efficient tissue-material interactions, and motivates further examination of this approach to optimize tissue-specific adhesion.

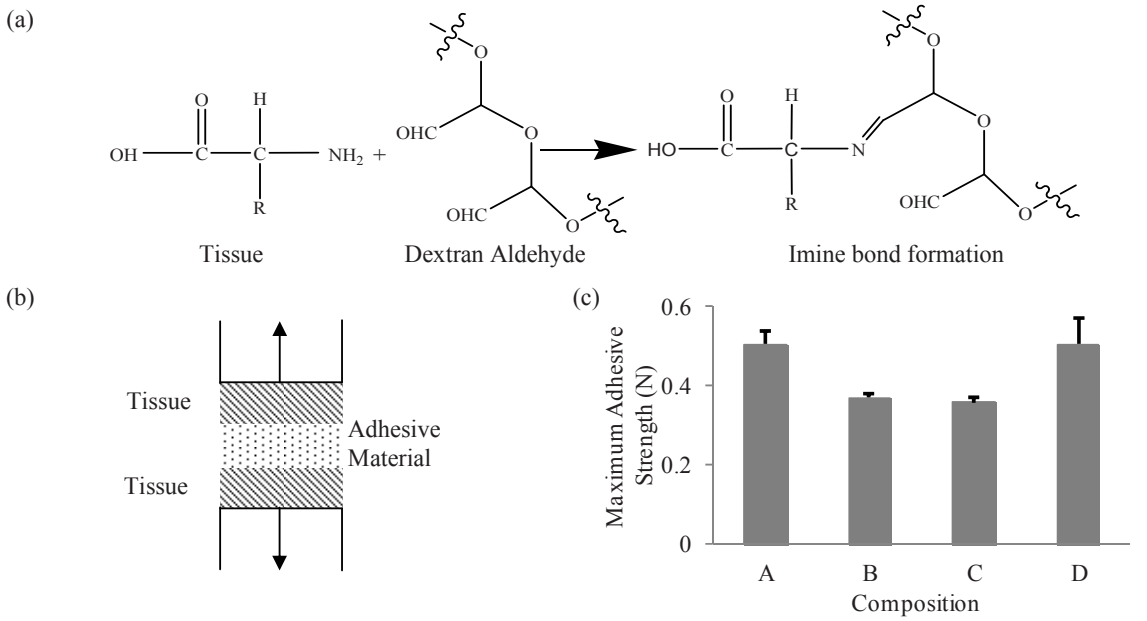

Fig. 3. (a) Imine bond formation between tissue and dextran aldehyde; (b) Schematic of tissue-material interface for adhesive strength measurement; (c) Aldehyde groups distribution index alters tissue-material adhesive strength.

Variation in reactive chemical groups distributions is hypothesized to differentially affect adhesion to other tissue types, as they exhibit different surface amine distributions for adhesive bond formation. Moreover, subsequent studies of the effects of aldehyde groups distribution index on biocompatibility, swelling and erosion kinetics, and adhesive interfacial morphology are required to fully explore the proposed method of optimizing tissue-specific adhesive materials.

Acknowledgements

This work was supported by the National Science Foundation/EPSCoR Grant (EPS-0903795).

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